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# Level 1 Biological Testing Assessment and Data Formatting

Litton Bionetics, Inc.
Kensington, MD

Prepared for

Industrial Environmental Research Lab.
Research Triangle Park, NC

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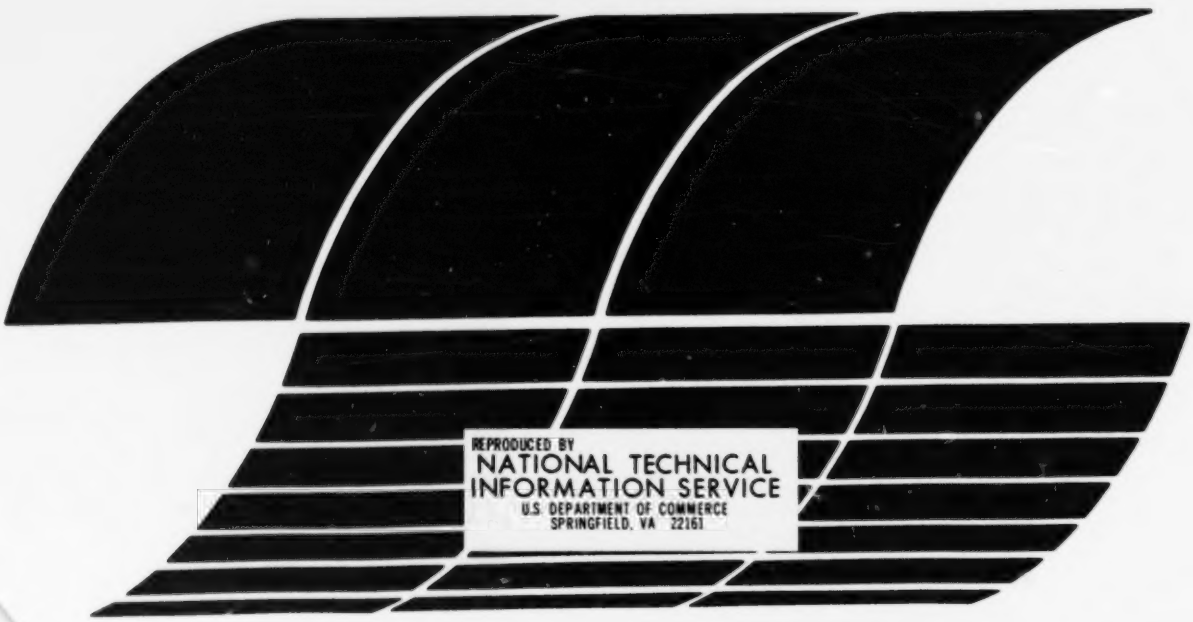
# Level 1 Biological Testing Assessment and Data Formatting

## Interagency Energy/Environment R&D Program Report



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# Level 1 Biological Testing Assessment and Data Formatting

by

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Contract No. 68-02-2681
Task No. 401
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EPA Project Officer: Raymond G. Merrill

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Research Triangle Park, NC 27711

Prepared for

U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
Washington, DC 20460

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## ABSTRACT

A scheme was developed for the comparison of Health Effect and Ecological Bioassay Assessment Data. The scheme was based on the assumption that each test method had a maximum dose (concentration) which could be reliably applied and that effects based on fractions of the maximum applicable dose (MAD) can be designated as degrees of toxicity. The levels of toxicity based on fractions of the MAD are given as high, moderate, low, and nondetectable (i.e no effect detected at the MAD).

Employing this scheme, data from three pilot studies including water effluent samples, fluidized bed combusion samples, and coal gasification samples were examined.

TABLE OF CONTENTS


| | | Page No. |
|---|---|---|
| ABSTRACT | | ii |
| LIST OF FIGURES | | iv |
| LIST OF TABLES | | iv |
| I. | INTRODUCTION | 1 |
| II. | DEFINITION OF HIGH, MEDIUM, LOW, AND NONDETECTABLE TOXICITY | 2 |
| III. | DATA TRANSITION FORMS | 4 |
| IV. | ABBREVIATIONS USED | 13 |
| V. | APPLICATION OF THE MAD CONCEPT FOR ASSIGNMENT OF TOXIC LEVELS IN PILOT STUDIES | 14 |
| VI. | DATA ORIGIN | 28 |
| VII. | AMES TEST DATA INTERPRETATION CRITERIA | 29 |
| VIII. | DATA CONVERSION SHEETS FOR TABLES | 32 |

## LIST OF FIGURES


| No. | | Page No. |
|---|---|---|
| 7.1 | Ames Test | 10 |
| 7.2 | *In Vitro* Cytotoxicity Assays | 11 |
| 7.3 | Rodent Toxicity Assay | 12 |


## LIST OF TABLES


| No. | | Page No. |
|---|---|---|
| 1. | Definition of Effectiveness Catagories | 3 |
| 2. | Primary Data Transititon Form | 5 |
| 3. | Health Effects Critical Data Summary Form: Mutagenicity | 6 |
| 4. | Health Effects Critical Data Summary Form: Toxicity | 7 |
| 5. | Ecological Effects Critical Data Summary Form | 8 |
| 6. | Bioassay Summary | 9 |
| 7. | Bioassay Summary Table | 16 |
| 8. | Health Effects Critical Data Summary Form: Mutagenicity | 17 |
| 9. | Ecological Effects Critical Data Summary Form | 18 |
| 10. | Health Effects Critical Data Summary Form Toxicity | 19 |
| 11. | Bioassay Summary Table | 20 |
| 12. | Health Effects Critical Data Summary Form: Mutagenicity | 21 |
| 13. | Health Effects Critical Data Summary Form: Toxicity | 22 |
| 14. | Ecological Effects Critical Data Form | 23 |
| 15. | Bioassay Summary Table | 24 |
| 16. | Health Effects Critical Data Summary Form: Mutagenicity | 25 |
| 17. | Health Effects Critical Data Summary Form: Toxicity | 26 |
| 18. | Ecological Effects Critical Data Summary Form | 27 |

# I. INTRODUCTION

This report presents a system to convert results from Level 1 health effects, bioassays, and selected Level 1 ecological bioassays into a uniform format. This format is structured so that data can be converted from original study reports into nondetectable, low, moderate, and high designations. The evaluation of raw data and development of the final toxicity definitions require a set of talbes and data transition sheets that provide a uniform method of data summarization.

The approach is based on the following rationale:

- Biological activity measured by lethality has sufficient, common phenomena to produce meaningful comparisons. The Ames Salmonella assay measures gene revertants rather than toxicity, but its results can be grouped in a similar fashion.

- Each assay, regardless of the type of response measured, has a maximum applicable dose above which the test data are virtually impossible to interpret because of nonspecific responses of the test organisms.

- A structure is needed for data assessment that can categorize toxicity data from diverse sources into a series of similar comparative categories. Without this, the comparison of these diverse assays would be difficult, and subject to variable interpretations.

- The categories low, moderate, and high are sufficiently broad to encompass normal variability and species differences, yet are narrow enough to provide meaningful categories for decision making.

- Level 1 testing is designed to rank sampling sites for further studies and is not intended for making human risk estimates.

Environmental assessment requires evaluation of data derived from chemistry, engineering, and biology. This data formatting program was developed to permit biological data to easily fit into the evaluation process without requiring the user to be an expert in numerous biological disciplines.

The data forms suggested here will be revised as Level 1 procedures are changed. Consult the most recent revision of "IERL-RTP Procedures Manual: Level 1 Biological Tests for Environmental Assessment" for current forms.

## II. DEFINITION OF HIGH, MEDIUM, LOW, AND NONDETECTABLE TOXICITY

Definition of the above classes of toxicity would be as follows:

Nondetectable--no significant response as determined by a preestablished set of criteria. Toxicants could be present in a sample below the level of detectability (MDC) for the assay.

Low--a significant response or an LD50, LC50, or EC50 value ranging from the maximum dose applicable to the assay system (defined as MAD and established in advance) to one-tenth of that value. For example, if it is established that 10 g/kg will define the upper limit of sample application to rats in the acute rodent toxicity test, then any sample which has an LD50 value of 10 g/kg down to a concentration of 1 g/kg will be defined as having low toxicity.

Medium--a significant response or an LD50, LC50, or EC50 value ranging from a concentraton less than the lower limit of the "low" toxicity (1/10 of the MAD) to 1/100 of the MAD. Using the same illustration, an LD50 (EC50) obtained at a concentration ranging from 0.1 g/kg to 1.0 g/kg in the rodent toxicity assay will be considered as moderate.

High--a significant response or an LD50 (EC50) value less than 1/100 of the MAD for the particular assay. Thus, any sample with an LD50 less than 0.1 g/kg in the rodent assay will be considered to have high toxicity. A summary of the toxicity levels and their associated definitions are shown in Table 1.

The degree of sample concentration will be factored into the calculation procedure and will reduce the level of toxicity accordingly.

Figures 1 to 3 illustrate the relationship of critical data (e.g. LD50) to high, moderate, low and nondectable designations.

The MAD values are taken from current protocols used in conducting these studies. The values proposed for MAD in Level 1 screening were established from:

- pilot study data developed from Level 1 testing,
- scientific publications dealing with uniform testing methods (Science, 203:563, 1979), and
- standard operating procedures defined by testing laboratories experienced in conducting Level 1 bioassays.

TABLE 1

DEFINITION OF EFFECTIVENESS CATEGORIES

| ASSAY | ACTIVITY MEASURED | UNITS | MAD | RANGE OF CONCENTRATION OR DOSAGE | | | |
|---|---|---|---|---|---|---|---|
| | | | | HIGH | MODERATE | LOW | NOT DETECTABLE |
| Ames Test | Mutagenesis* | mg/plate | 5 | <0.05 | 0.05-0.5 | 0.5-5 | ND at >5 |
| | | µl/plate | 50 | <0.5 | 0.5-5.0 | 5-50 | ND at >50 |
| RAM/WI-38 and CHO TOXICITY | Lethality EC50 | mg/ml | 1 | <0.01 | 0.01-0.1 | 0.1-1 | ND at >1 |
| | | µl/ml | 600 | <6.0 | 6.0-60 | 60-600 | ND at >600 |
| | | µl/ml | 20 ** | | | | ND at >20 |
| RODENT TOXICITY | Lethality LD50 | gm/kg | 10 | <0.1 | 0.1 -1 | 1 -10 | ND at >10 |
| Aquatic Tests Algae | Growth Inhibition EC50 | gm/liter | 1 | <0.01 | 0.01-0.1 | 0.1-1 | ND at >1 |
| | | % | 100 | <20 | 20-75 | 75-100 | ND at >100 |
| Fish | Lethality LC50 | gm/liter | 1 | <0.01 | 0.01-0.1 | 0.1-1 | ND at >1 |
| | | % | 100 | <20 | 20-75 | 75-100 | ND at >100 |
| Invertebrate | Lethality LC50 | gm/liter | 1 | <0.01 | 0.01-0.1 | 0.1-1 | ND at >1 |
| | | % | 100 | <20 | 20-75 | 75-100 | ND at >100 |

Abbreviations:
MAD = Maximum Applicable Dose (Technical Limitations)
LD50 = Calculated Dosage Expected to Kill 50% of Population
LC 50 = Calculated Concentration Expected to Kill 50% of Population
EC50 = Calculated Concentration Expected to Produce Effect in 50% of Population
ND = Not Detectable
*Negative response at 5 mg/plate or at level of toxicity is given as ND.
Positive response requires calculation of minimum effective concentration (MEC) to produce a positive mutagenic response. H, M and L designations are made from MEC values of positive agents.

**Volumes used for solvent exchange samples (This maximum keeps DMSO below level of toxicity).

## III. DATA TRANSITION FORMS

In order to ensure uniform translation of raw data to definitions of toxicity, three intermediate forms were developed.

The Primary Data Transition Form (Table 2) records the basic information from a test by sample. The type of test, is respective MAD, and the calculated active dose are recorded on this table. Since the Ames Salmonella assay is a special case, the LC50 value is not directly applicable. The Ames test is evaluated as either positive (+) or negative (-). If it is (-), the level of activity is not as significant a the fact that the sample was not mutagenic. If the sample was (+), however, it is possible to assess the level of activity by defining the lowest concentration of the test sample at which mutagenicity could be detected (activity is defined by the preestablished criteria suggested in Appendix A).

The primary form is also used to record testing methods which concentrate a sample after receipt, such as solvent exchange. The concentration factor is the ratio of the total volume sampled to the final sample volume. There is also a place to record commments regarding the assay that provide backup documentation or explanations. In several tests (e.g., RAM), several parameters indicative of cell lethality are measured. Each of these should be recorded in the primary data transition form (Table 2) and the most sensitive parameter used to determine "Level of toxicity" for the Bioassay Summary (Table 6). Without some method of ranking each parameter, this is considered an adequate approach to uniform data interpretation.

The Primary Data Transition Forms are often used when results are submitted by more than one biological contractor. It is designed to provide a uniform base for assimilating data for transition to subsequent Level 1 formats.

Health and Ecological Effects Critical Data Summary Forms, (Tables 3, 4, and 5) combine the data from Level 1 tests conducted on samples from a single site. Tables 3 and 4 summarize reponses from health effects tests and Table 5 summarizes the response from aquatic tests.

Tables 2 to 5 contain the responses which are finally summarized for all Level 1 tests in Table 6. It is a summary of the various tests using only ND, L, M, and H response designations. Table 6 allows rapid ranking of the streams evaluated by the variety of tests used in Level 1.

Table 2

PRIMARY DATA TRANSITION FORM

Assay: _____ Sample: _____

MAD: _____ Date Processers Name: _____

Results:

LD50 [EC50] Value[a] _____ [or] Ames Response _____

MEC[b] _____

Approximate Concentration Factor _____

Level of Toxicity[c] _____

Comments:[d]

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration [if response is positive].

[c]See Table 1 for definition of toxicity levels.

[d]May include;

Salmonella strains responding to a mutagenic substance.
Deviations from standard protocols.
Toxic signs in rodent assay.
Procedural problems.

# TABLE 3

HEALTH EFFECTS CRITICAL DATA
SUMMARY FORM: MUTAGENICITY

Contract No. _____ Site Sampled _____

Technical Directive or Project No. _____

| Sample Identification | Salmonella Plate Assay | |
|---|---|---|
| | Response[a] | LRPC[b] |
| | | |
| | | |
| | |

TABLE 4

HEALTH EFFECTS CRITICAL DATA SUMMARY FORM: TOXICITY

Contract ID. _____ Technical Directive or Project ID. _____ Site ID. _____

| Sample Identification | CHO Toxicity | RAM Cell Toxicity | | | WI-38 Cell Toxicity | | | Rodent Toxicity | |
|---|---|---|---|---|---|---|---|---|---|
| | LC50 | Viability[a] | ATP[b] | Protein[c] | Viability[a] | ATP[b] | Protein[c] | LD50 | Toxic Signs[d] |
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[a] Viability Index (see EPA 600/7-77-043)
[b] ATP (see EPA 600/7-77-043 for derivation)
[c] Protein (see EPA 600/7-77-043 for derivation)
[d] Toxic signs are identified in a numbered list in EPA 600/7-77-043. Only the number is reported here

# TABLE 5

ECOLOGICAL EFFECTS CRITICAL DATA SUMMARY FORM

Contract No. _____ Technical Directive or Project No. _____ Site sampled _____

| Sample Identification | Fresh Water Toxicity Tests[a] | | | Marine Toxicity Tests[a] | | |
|---|---|---|---|---|---|---|
| | Fish LC50 | Invertebrate LC50 | ALGAL EC50 | Fish LC50 | Invertebrate LC50 | ALGAL EC50 |
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[a]The derivations of all test values are explained in the text of the report of the Level 1 manual.

# TABLE 6

## BIOASSAY SUMMARY

Technical Directive or Project No. _____

Contract No. _____

| Sample Identification | Salmonella | Ram Cytotoxicity | WI-38 Cytotoxicity | CHO Cytotoxicity | Rodent Toxicity | Fish | Invertebrate | Algal | Fish | Invertebrate | Algal | Plant Stress Ethylene | Soil Test | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Fresh Water | | | Marine | | | | |
| | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
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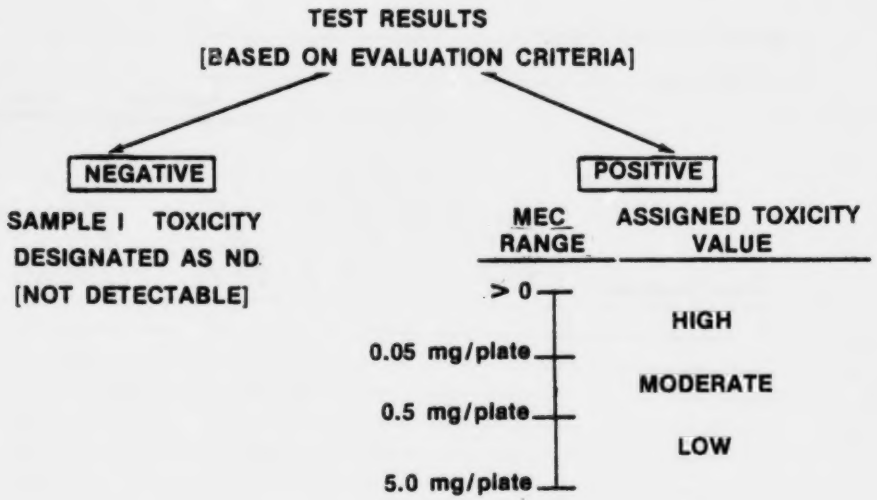
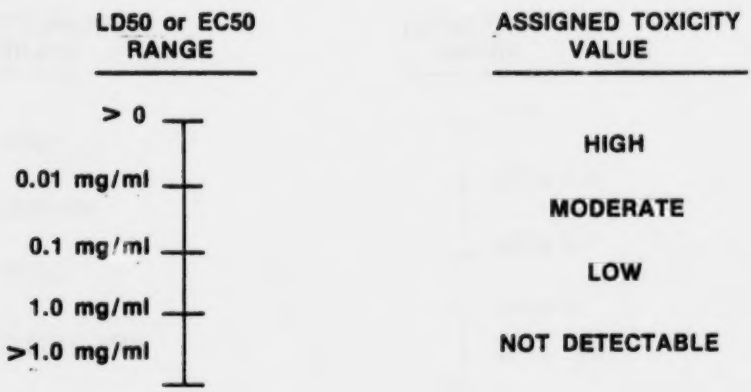


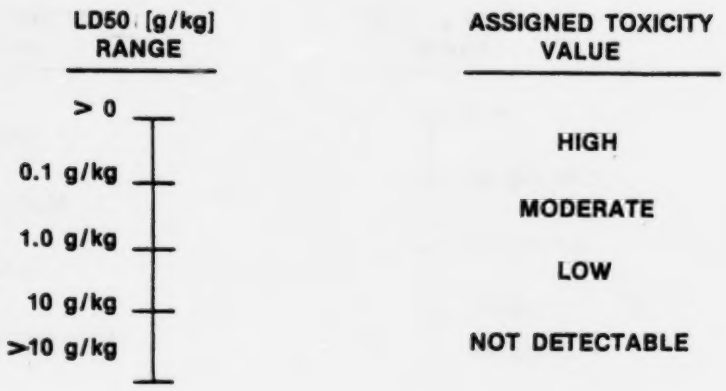

The Maximum Applicable Dose [MAD] is set at 5 mg of substance per plate.

Figure 7.1 AMES TEST




1. CHO cells use viability index [EC50].
2. For RAM or WI-38 assays use the EC50 value from the most sensitive parameter [viability, ATP, Protein].

Figure 7.2 IN VITRO CYTOTOXICITY ASSAYS




**The Maximum Applicable Dose [MAD] is set at 10 grams of substance per kilogram of rat body weight.**

**Figure 7.3 RODENT TOXICITY ASSAY**

## IV. ABBREVIATIONS USED

MDC = Minimum Detectable Concentration
MAD = Maximum Applied Dose
LD50 = Lethal Dose to 50% of the Test Organisms
LC50 = Lethal Concentration for 50% of the Test Organisms
EC50 = Effective Concentration for 50% of the Test Organisms
MEC = Minimum Effective Concentration
LRPC = Lowest Recorded Positive Concentration

## V. APPLICATION OF THE MAXIMUM APPLIED DOSE CONCEPT FOR ASSIGNMENT OF TOXIC LEVELS

By examination of already existing pilot study reports (supplied by EPA), test results were formatted using the methods described in this report. Numerous parameters could be considered in this data transfer, but we proposed a simple route relying primarily on LD50, LC50, EC50, or MEC. The data analyses given in reports submitted to EPA by various testing laboratories were amenable to this approach, and data conversion into toxic levels was accomplished readily.

Summaries of results from the application of this approach are shown in Tables 7, 11, and 15.

## Legend to Tables 7, 11, and 15

These tables are a summary of EPA submitted data for Level 1 Pilot Study Bioassays.

The data were obtained from bioassays of various water effluents (Table 7), fluidized bed combustion samples (Table 11), and coal gasification procedures (Table 15).

Toxicity is defined as:

H = High

M = Moderate

L = Low

ND = Nondetectable

Tables 8, 9, 10, and 12, 13, 14, and 16, 17, 18 are intermediate summary tables. Reported values are given as concentration based on Table 1.

TABLE 7
BIOASSAY SUMMARY

**Technical Directive or Project No.** Water Effluent Study

**Contract No.** Pilot Study Results

| Sample Identification | Salmonella | Ram Cytotoxicity | WI-38 Cytotoxicity | CHO Cytotoxicity | Rodent Toxicity | Fish | Invertebrate | Algal | Fish | Invertebrate | Algal | Plant Stress Ethylene | Soil Test | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh Water | | | | | | | | | Marine | | | | |
| A2 | ND | | | | ND | H | H | L | M | M | | | | |
| B2 | ND | | | | ND | ND | ND | M | ND | ND | ND | | | |
| C2 | ND | M | | | ND | M | M | ND | M | L | L | | | |
| D2 | ND | | | | ND | | | | | | | | | |
| E2 | ND | | | | ND | ND | H | H | | | M | | | |
| F2 | ND | | | | ND | ND | L | | ND | ND | M | | | |
| G2 | ND | | | | ND | M | M | ND | ND | ND | M | | | |
| H2 | ND | | | | ND | | | L | | | | | | |
| J2 | ND | | | | ND | ND | ND | ND | | | | | | |
| K2 | ND | | | | ND | ND | ND | ND | ND | ND | L | | | |
| L2 | ND | L | L | M | ND | M | M | M | L | ND | H | | | |
| M2 | ND | L | ND | ND | ND | ND | M | ND | | | | | | CHO >200 µl |
| N2 | ND | H | L | L | ND | M | H | H | M | M | H | | | |
| P2 | ND | | | | ND | ND | ND | M | | | H | | | |
| R2 | ND | ND | ND | M | ND | H | H | L | | | | | | |
| S2 | ND | | | | ND | ND | | ND | ND | ND | ND | | | |
| T2 | ND | | | | ND | M | ND | ND | M | | L | | | |
| U2 | ND | | | | ND | ND | H | ND | ND | ND | ND | | | |
| V2 | ND | | | | ND | M | H | ND | | | L | | | |
| W2 | ND | | | | ND | M | H | L | M | M | M | | | |
| X2 | ND | | | | ND | ND | ND | ND | ND | ND | ND | | | |
| Y2 | ND | | | | ND | ND | ND | ND | | | | | | |
| Z2 | ND | | | | ND | ND | M | H | | | | | | |

Data transition sheets for all results not shown in Section VIII.

ND = No Detectable Toxicity
L = Low Toxicity
M = Moderate Toxicity
H = High Toxicity

LBL-0468 3/79

TABLE 8

HEALTH EFFECTS CRITICAL DATA
SUMMARY FORM: MUTAGENICITY

Contract No. Pilot Site Sampled WE

Technical Directive or Project No. NA

| Sample Identification | Salmonella Plate Assay Response[a] | LRPC[b] |
|---|---|---|
| A2 | - | NA |
| B2 | - | NA |
| C2 | - | NA |
| D2 | - | NA |
| E2 | - | NA |
| F2 | - | NA |
| G2 | - | NA |
| H2 | - | NA |
| J2 | - | NA |
| K2 | - | NA |
| L2 | - | NA |
| M2 | - | NA |
| N2 | - | NA |
| P2 | - | NA |
| R2 | - | NA |
| S2 | - | NA |
| T2 | - | NA |
| U2 | - | NA |
| V2 | - | NA |
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| X2 | - | NA |
| Y2 | - | NA |
| Z2 | - | NA |
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[a] given as + or - based on criteria in Appendix A
[b] LRPC = lowest recorded positive concentration (NA for negative data)

TABLE 9

ECOLOGICAL EFFECTS CRITICAL DATA SUMMARY FORM

Contract No. Pilot Technical Directive or Project No. NA Site sampled WE

| Sample Identification | Fresh Water Toxicity Tests[a] | | | Marine Toxicity Tests[a] | | |
|---|---|---|---|---|---|---|
| | Fish LC50 | Invertebrate LC50 | ALGAL EC50 | Fish LC50 | Invertebrate LC50 | ALGAL EC50 |
| A2 | 19% | 9% | 76% | 60% | 25% | |
| B2 | >100% | >100% | 30% | >100% | >100% | >100% |
| C2 | 46.5% | 41.0% | >100% | 70% | >10J% | 90% |
| D2 | | | | | | |
| E2 | >100% | 7.8% | 2% | | | 25% |
| F2 | >100% | 81.7% | | >100% | >100% | 70% |
| G2 | 64.7% | 62.4% | | >100% | >100% | 59% |
| H2 | | | 96% | | | |
| J2 | > 100% | >100% | >100% | | | |
| K2 | >100% | >100% | >100% | >100% | >100% | 77% |
| L2 | 25.5% | 28.0% | 42.0% | >100% | >100% | 2% |
| M2 | >100% | 60% | >100% | | | |
| N2 | 48.8% | 10% | 2% | 40% | 25% | 2% |
| P2 | >100% | >100% | 43% | | | 9% |
| R2 | 16.5% | 8% | 93% | | | |
| S2 | >100% | | >100% | >100% | >>100% | >100% |
| T2 | 46.5% | >100% | >100% | 70% | | 70% |
| U2 | >100% | 12.1% | >100% | >100% | >100% | >100% |
| V2 | 36% | 9.4% | >100% | | | 94% |
| W2 | 55.2% | 6.3% | 94% | 56% | 25% | 50% |
| X2 | >100% | >100% | >100% | >100% | >100% | >100% |
| Y2 | >100% | >100% | >100% | | | |
| Z2 | >100% | 42.6% | 18% | | | |

[a]The derivations of all test values are explained in the test of the report of the Level I manual.

TABLE 10

HEALTH EFFECTS CRITICAL DATA SUMMARY FORM: TOXICITY

Contract ID. Pilot Technical Directive or Project ID. NA Site ID. WE

| Sample Identification | CHO Toxicity | RAM Cell Toxicity | | | WI-38 Cell Toxicity | | | Rodent Toxicity | |
|---|---|---|---|---|---|---|---|---|---|
| | LC50 | Viability[a] | ATP[b] | Protein[c] | Viability[a] | ATP[b] | Protein[c] | LD50 | Toxic Signs[d] |
| A2 | | | | | | | | 10g/kg | None |
| B2 | | | | | | | | 10g/kg | None |
| C2 | | | 33.5 µl | | | | | 10g/kg | None |
| D2 | | | | | | | | 10g/kg | None |
| E2 | | | | | | | | 10g/kg | None |
| F2 | | | | | | | | 10g/kg | None |
| G2 | | | | | | | | 10g/kg | None |
| H2 | | | | | | | | 10g/kg | None |
| J2 | | | | | | | | 10g/kg | None |
| K2 | | | | | | | | 10g/kg | None |
| L2 | 60 µl | | 357 µl | | | 300 µl | - | 10g/kg | None |
| M2 | >200 µl | 400 µl | | | >600 µl | >600 µl | >600 µl | 10g/kg | None |
| N2 | 75 µl | 13.3 µl | 175 µl | | | 200 µl | | 10g/kg | None |
| P2 | | | | | | | | 10g/kg | None |
| R2 | 37 µl | >600 µl | >600 µl | >600 µl | >600 µl | >600 µl | >600 µl | 10g/kg | None |
| S2 | | | | | | | | 10g/kg | None |
| T2 | | | | | | | | 10g/kg | None |
| U2 | | | | | | | | 10g/kg | None |
| V2 | | | | | | | | 10g/kg | None |
| W2 | | | | | | | | 10g/kg | None |
| X2 | | | | | | | | 10g/kg | None |
| Y2 | | | | | | | | 10g/kg | None |
| Z2 | | | | | | | | 10g/kg | None |

[a]Viability Index (see EPA 600/7-77-043)
[b]ATP (see EPA 600/7-77-043 for derivation)
[c]Protein (see EPA 600/7-77-043 for derivation)
[d]Toxic signs are identified in a numbered list in EPA 600/7-77-043. Only the number is reported here

TABLE 11
BIOASSAY SUMMARY

Technical Directive or Project No. Fluidized Bed Combustion (FBC)

Contract No. Pilot Study Data

| Sample Identification | Salmonella | Ram Cytotoxicity | WI-38 Cytotoxicity | CHO Cytotoxicity | Rodent Toxicity | Fresh Water: Fish | Fresh Water: Invertebrate | Fresh Water: Algal | Marine: Fish | Marine: Invertebrate | Marine: Algal | Plant Stress Ethylene | Soil Test | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spent Bed Leachate | | ND | | | ND | M | M | M | ND | ND | ND | | | |
| Dolomite Leachate | ND | ND | | | ND | ND | ND | ND | ND | L | ND | | | |
| Flyash Leachate | | ND | | | ND | ND | ND | ND | ND | M | ND | | | |
| Coal Leachate | ND | | | | | L | M | H | M | M | ND | | | |
| 3μ Cyclone Particles | L | ND | | | | | | | | | | | | |
| 1μ Cyclone Particles | L | L | | | | | | | | | | | | |
| Dolomite | ND | ND | | | ND | | | | | | | | | |
| Flyash | ND | ND | | | ND | | | | | | | | | |
| Spent Bed | ND | ND | | | ND | | | | | | | | | |
| Slurry | | L | | | ND | M | M | H | M | H | M | | | |
| Cyclone Discard Leachate | | | | | | ND | ND | ND | | | | | | |

ND = No Detectable Toxicity
L = Low Toxicity
M = Moderate Toxicity
H = High Toxicity

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TABLE 12

HEALTH EFFECTS CRITICAL DATA
SUMMARY FORM: MUTAGENICITY

Contract No. Pilot Site Sampled FBC

Technical Directive or Project No. NA

| Sample Identification | Salmonella Plate Assay Response[a] | LRPC[b] |
|---|---|---|
| 1μ Cyclone | + | 2 mg/plate |
| 3μ Cyclone | + | 2 mg/plate |
| Dolomite | - | NA |
| Flyash | - | NA |
| Spent Bed Dolomite | - | NA |
| Dolomite Leachate | - | NA |
| Coal Leachate | - | NA |

[a] given as + or - based on criteria in Appendix A
[b] LRPC = lowest recorded positive concentration (Negative data = NA)

TABLE 13

HEALTH EFFECTS CRITICAL DATA SUMMARY FORM: TOXICITY

Contract ID. Pilot Technical Directive or Project ID. NA Site ID. FBC

| Sample Identification | CHO Toxicity LC50 | RAM Cell Toxicity Viability[a] | RAM Cell Toxicity ATP[b] | RAM Cell Toxicity Protein[c] | WI-38 Cell Toxicity Viability[a] | WI-38 Cell Toxicity ATP[b] | WI-38 Cell Toxicity Protein[c] | Rodent Toxicity LD50 | Rodent Toxicity Toxic Signs[d] |
|---|---|---|---|---|---|---|---|---|---|
| 3μ Cyclone | | >1000μg | >1000μg | >1000μg | | | | >10g/kg | NONE |
| Fly Ash | | >1000μg | >1000μg | >1000μg | | | | >10g/kg | NONE |
| Dolomite | | >1000μg | >1000μg | >1000μg | | | | >10ml/kg | NONE |
| Fly Ash Leachate | | > 600μl | > 600μl | > 600μl | | | | >10ml/kg | NONE |
| Slurry | | > 600μl | > 146μg | > 600μl | | | | >10ml/kg | NONE |
| Spent Bed Leachate | | > 600μl | > 600μl | > 600μl | | | | | |
| 1 Cyclone | | >1000μg | > 596μg | >1000μg | | | | >10g/kg | NONE |
| Spent Bed Dolomite | | >1000μg | >1000μg | >1000μg | | | | >10ml/kg | NONE |
| Dolomite Leachate | | > 600μl | > 600μl | > 600μl | | | | | |

[a] Viability Index (see EPA 600/7-77-043)
[b] ATP (see EPA 600/7-77-043 for derivation)
[c] Protein (see EPA 600/7-77-043 for derivation)
[d] Toxic signs are identified in a numbered list in EPA 600/7-77-043. Only the number is reported here

TABLE 14

ECOLOGICAL EFFECTS CRITICAL DATA SUMMARY FORM

Contract No. Pilot  Technical Directive or Project No. NA  Site sampled FBC

| Sample Identification | Fresh Water Toxicity Tests[a] | | | Marine Toxicity Tests[a] | | |
|---|---|---|---|---|---|---|
| | Fish LC50 | Invertebrate LC50 | ALGAL EC50 | Fish LC50 | Invertebrate LC50 | ALGAL EC50 |
| Spent Bed Leachate | 25% | 40.9% | 45% | >100% | >100% | >100% |
| Dolomite Leachate | >100% | >100% | >100% | >100% | 74.8% | 56% |
| Flyash Leachate | >100% | >100% | >100% | >100% | 72.5% | >100% |
| Coal Leachate | 92% | 70.9% | 19% | 11.5% | 66% | >100% |
| Slurry | 67.9% | 13.6% | 9% | 70.5% | 18% | 62% |
| Cyclone Discard Leachate | >100% | >100% | >100% | | | |
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[a]The derivations of all test values are explained in the text of the report of the Level 1 manual.

TABLE 15
BIOASSAY SUMMARY

Technical Directive or Project No. Coal Gasification (CG)

Contract No. Pilot Study Data

| Sample Identification | Salmonella | Ram Cytotoxicity | WI-38 Cytotoxicity | CHO Cytotoxicity | Rodent Toxicity | Fresh Water: Fish | Fresh Water: Invertebrate | Fresh Water: Algal | Marine: Fish | Marine: Invertebrate | Marine: Algal | Plant Stress Ethylene | Soil Test | Notes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Separator Liquor* | ND | M | M | | | H | H | H | H | H | H | | | |
| 590-3 (XAD-2) | L | H | | | | | | | | | | | | |
| 655-3 (XAD-2) | ND | M | | | | | | | | | | | | |
| 655-5/6 (XAD-2) | | H | H | | | | | | | | | | | |
| 590-5/6 (XAD-2) | | H | H | | | | | | | | | | | |
| Coal | ND | ND | | | ND | | | | | | | | | |
| Coal Dust | | ND | | | | | | | | | | | | |
| Cyclone Dust | ND | ND | | | ND | | | | | | | | | |
| Separator Tar | M | L | M | | ND | | | | | | | | | |
| Ash | ND | L | | | ND | | | | | | | | | |

ND = No Detectable Toxicity
L = Low Toxicity
M = Moderate Toxicity
H = High Toxicity

*Data from all samples designated Separator Liquor combined.

TABLE 16

HEALTH EFFECTS CRITICAL DATA
SUMMARY FORM: MUTAGENICITY

Contract No. Pilot Site Sampled CG

Technical Directive or Project No. NA

| Sample Identification | Salmonella Plate Assay Response[a] | LRPC[b] |
|---|---|---|
| 590 | + | 1 mg/plate |
| 655 | - | NA |
| Coal | - | NA |
| Ash | - | NA |
| Cyclone | - | NA |
| Separator Tar | - | 0.01 mg/plate |
| Separator Liquor | - | NA |

[a] given as + or - based on criteria in Appendix A
[b] LRPC = lowest recorded positive concentration (Negative data = NA)

TABLE 17

HEALTH EFFECTS CRITICAL DATA SUMMARY FORM: TOXICITY

Contract ID. Pilot Technical Directive or Project ID. NA Site ID. CG

| Sample Identification | CHO Toxicity LC50 | RAM Cell Toxicity Viability[a] | RAM Cell Toxicity ATP[b] | RAM Cell Toxicity Protein[c] | WI-38 Cell Toxicity Viability[a] | WI-38 Cell Toxicity ATP[b] | WI-38 Cell Toxicity Protein[c] | Rodent Toxicity LD50 | Rodent Toxicity Toxic Signs[d] |
|---|---|---|---|---|---|---|---|---|---|
| 590-3 | | - | 5.3 µl | - | | | | | |
| 655-3 | | 21.2 µl | - | - | | | | | |
| Coal | | >1000 µl | >1000 µl | >1000µl | | | | >10g/kg | NONE |
| 655-5/6 | | < 6.0 µl | - | - | <6.0 µl | - | - | | |
| 590-5/6 | | < 6.0 µl | - | <6.0 µl | <6.0 µl | | | | |
| Coal Dust | | >1000 µg | >1000 µg | >1000 µg | | | | | |
| Separator Tar | | 350 µg | - | - | | | | | |
| Cyclone Dust | | >1000 µg | >1000 µg | >1000 µg | | | | >10g/kg | NONE |
| Separator Liquor | | 12 µl | - | - | 7.4 µl | - | - | >10g/kg | NONE |
| Ash | | >300 µg | >300 µg | >300 µg | | | | >10g/kg | NONE |
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[a] Viability Index (see EPA 600/7-77-043)
[b] ATP (see EPA 600/7-77-043 for derivation)
[c] Protein (see EPA 600/7-77-043 for derivation)
[d] Toxic signs are identified in a numbered list in EPA 600/7-77-043. Only the number is reported here

TABLE 18

ECOLOGICAL EFFECTS CRITICAL DATA SUMMARY FORM

Contract No. Pilot Technical Directive or Project No. NA Site sampled CG

| Sample Identification | Fresh Water Toxicity Tests[a] Fish LC50 | Invertebrate LC50 | ALGAL EC50 | Marine Toxicity Tests[a] Fish LC50 | Invertebrate LC50 | ALGAL EC50 |
|---|---|---|---|---|---|---|
| Separator Liquor | <1.0% | <1.0% | <1.0% | <1.0% | <1.0% | <1.0% |
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[a]The derivations of all test values are explained in the text of the report of the Level 1 manual.

VI. DATA ORIGIN

| SOURCE | CONTRACT # | DATE |
|---|---|---|
| Northrop Services, Inc. | 68-02-2566 | 1978 |
| EG&G Bionomics | - | 1977 |
| SRI | 68-01-2458 | 1977 |
| SRI | - | 1978 |
| Battelle | 68-02-2138 | 1977 |
| Litton Bionetics, Inc. | - | 1977 |
| Monsanto Res. Corp. | 68-02-1874 | 1978 |

## VII. AMES TEST DATA INTERPRETATION CRITERIA

### A. Evaluation Criteria

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay (Ames et al., 1975). Because the test chemical and the cells are incubated in the overlay for 2 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 days.

#### 1. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plate is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over 2 or 3 log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

#### 2. Dose-Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose if far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

3. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assay. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

4. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

a. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

b. Strains TA-98 and TA-100

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strain TA-98 is considered to be mutagenic. For these strains, the dose-response increase should start at approximately the solvent control value.

c. Pattern

Because TA-1535 and TA-100 are both derived from the same parental strain (G-46) and because TA-1538 and TA-98 are both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general, the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g., TA-1537, responds to a mutagen in nonactivation tests, it will generally do so in activation tests. (The converse of this relationship is not expected). While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

d. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive data lose significance.

The preceding criteria are not absolute, and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteral for evaluation can be more firmly established.

5. Relationship Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/Microsome Plate Test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two end points. The results of comparative tests on 300 chemicals by McCann et al. (1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

B. References

Ames, B.N., McCann, J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res. 31, 347-364.

McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975). Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. 72, 5135-5139.

## VIII. DATA CONVERSION SHEETS FOR TABLES 7, 11, AND 15

### Abbreviations of Sample Source

FBC = Fluidized Bed Combustion
CG = Coal Gasification
WE = Water Effluent Samples

### Organisms Employed in Aquatic Tests

- Freshwater

| | | | |
|---|---|---|---|
| Fish | Fathead | Minnow | (*Pimephates promelas*) |
| Algae | *Selenastrum capricornutum* | | |
| Invertebrate | *Daphnia* | *Magna* | |

- Marine

| | | | |
|---|---|---|---|
| Fish | Sheepshead Minnow | | (*Cyprinodon variegatus*) |
| Algae | *Skeletonema costatum* | | |
| Invertebrate | Grass | Shrimp | (*Palaemonetes pugio*) |

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** Grass shrimp **SAMPLE:** Coal leachate

**MAD:** 100%

**RESULTS:**

**LD50 [EC50] VALUE**[a] 96 hour LC50 = 66% **[or] AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Moderate

**COMMENTS:**

24 hr toxicity = 75% effluent

48 hr toxicity = 72.5% effluent. Diss. $O_2$ = 42% of saturation

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration [if response is positive].

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** Grass shrimp **SAMPLE:** Flyash leachate

**MAD:** 100%

**RESULTS:**

**LD50 [EC50] VALUE**[a] LC50 = 72.5% E at 96 hr. **[or] AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Moderate

**COMMENTS:**

24 and 48 hr. LC50's = 75% E

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration [if response is positive].

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** FBC

**ASSAY:** Grass Shrimp **SAMPLE:** Dolomite Leachate

**MAD:** 100% E

**RESULTS:**

**LD50 (EC50) VALUE**[a] LC50 = 74.8% E at 96 hr. **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] L

**COMMENTS:**

24 and 48 hr. LC50's = 100% E.

E = Effluent

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** FBC

**ASSAY:** Grass Shrimp **SAMPLE:** N 9661 (Slurry)

**MAD:** 100% E

**RESULTS:**

**LD50 (EC50) VALUE**[a] LC50 = 18% at 96 hr. **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] High

**COMMENTS:**

48 hr. LC50 = 34.5% Effluent.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** Grass shrimp **SAMPLE:** Spentbed leachate

**MAD:** 100% Effluent

**RESULTS:**

**LD50 (EC50) VALUE**[a] Not Toxic >100% (or) **AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] ND

**COMMENTS:** Not detectable

Diss. $O_2$ = 55% of saturation at all test levels.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM**

FBC

**ASSAY:** Fish toxicity **SAMPLE:** Dolomite leachate

**MAD:** 100%

**RESULTS:**

**LD50 (EC50) VALUE**[a] Not toxic at 100% **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] ND

**COMMENTS:** Not detected

No toxicity after 96 hr. exposure. Diss. $O_2$ after 96 hrs. ranged from 26% of saturation in 10% of effluent to 48% of saturation in the control and 100% effluent.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM**

FBC

**ASSAY:** Fish toxicity **SAMPLE:** N 9 661 (Slurry)

**MAD:** 100%

**RESULTS:**

**LD50 (EC50) VALUE**[a] LC50 = 70.5%E at 19 hr. **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Moderate

**COMMENTS:**

Not toxic at 24 and 48 hr exposures.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine Fish toxicity SAMPLE: Coal leachate

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] LC50 = 71.5% effluent (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Moderate

COMMENTS:

All three time periods: 24-, 48- and 96 hrs. gave LC50's of 71.5% effluent.
Diss. $O_2$ = 39% - 62% of saturation.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine Fish toxicity SAMPLE: Flyash leachate

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] Not toxic at 96 hrs. (>100%) (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS: Not detectable

Diss $O_2$ = 48% of saturation in all tests.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** FBC

**ASSAY:** Marine Fish toxicity **SAMPLE:** Spent bed leachate

**MAD:** 100%

**RESULTS:**

**LD50 [EC50] VALUE**[a] Not toxic at 100% **[or] AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] ND

**COMMENTS:** Not detected

No mortality after 96 hr. exposure. Diss. $O_2 \geq 57\%$ of saturation in all test levels.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration [if response is positive]

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Freshwater Algal/DAPHNIA/Fish SAMPLE: Dolomite Leachate

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] > 100%/> 100%/ > 100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0 / 0 / 0

LEVEL OF TOXICITY[c] ND / ND / ND

COMMENTS: Not detectable

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Freshwater Algal/DAPHNIA/Fish SAMPLE: Spent Bed Leachate

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] ~ 45%/40.9/25 (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0 / 0 / 0

LEVEL OF TOXICITY[c] Moderate/Moderate/Moderate

COMMENTS:

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** FBC

**ASSAY:** Freshwater Algal/DAPHNIA/Fish **SAMPLE:** Flyash Leachate

**MAD:** 100%

**RESULTS:**

**LD50 (EC50) VALUE**[a] > 100%/ > 100%/ > 100% **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0 / 0 / 0

**LEVEL OF TOXICITY**[c] ND / ND / ND

**COMMENTS:** Not detectable

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** FBC

**ASSAY:** Freshwater Algal/DAPHNIA/Fish **SAMPLE:** Coal Leachate

**MAD:** 100%

**RESULTS:**

**LD50 (EC50) VALUE**[a] 19% / 70.9 / 92% **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0 / 0 / 0

**LEVEL OF TOXICITY**[c] High / Moderate / Low

**COMMENTS:**

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** Freshwater Algal/DAPHNIA/Fish **SAMPLE:** Scrubber Slurry

**MAD:** 100%

**RESULTS:**

**LD50 [EC50] VALUE**[a] 9% / 31.6% / 67.9% **[or] AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0 / 0 / 0

**LEVEL OF TOXICITY**[c] High / Moderate / Moderate

**COMMENTS:**

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration [if response is positive]

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine Alga SAMPLE: Spent bed leachate

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] Not Toxic >100% [or] AMES RESPONSE

MEC[b]

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] NO

COMMENTS:

Optical density increased by effluent.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine Alga SAMPLE: Coal leachate

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] Unaltered effluent Not toxic [or] AMES RESPONSE

MEC[b]

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS: Not detectable
Filtered effluent gave EC50's at 98, 39 and 38% effluent for 4, 8 and 12-day exposures.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine Alga SAMPLE: N 9661

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] EC50 62% of effluent at 4 days (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Moderate

COMMENTS:

The EC50's reported for unaltered effluent were 62% for 4 days and 66% for 12 days exposure. Filtered effluent was more active that unaltered effluent with EC50's of 22 and 15% for 4 and 12 days.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Marine alga SAMPLE: Dolomite leachate

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] EC50 = 56% effluent (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Moderate

COMMENTS:

Filtered effluent gave EC50's as 19% and 24% for 8 and 12 days respectively.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** FBC

**ASSAY:** Marine Alga **SAMPLE:** Flyash leachate

**MAD:** 100% effluent

**RESULTS:**

**LD50 (EC50) VALUE**[a] Not toxic >100% **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] ND

**COMMENTS:** Not detectable

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** Rodent toxicity **SAMPLE:** Fly Ash

**MAD:** 10 g/kg

**RESULTS:**

**LD50 (EC50) VALUE**[a] > 10 g/kg **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**

No toxic sign reported.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** Rodent toxicity **SAMPLE:** Dolomite

**MAD:** 10 g/kg

**RESULTS:**

**LD50 (EC50) VALUE**[a] 10 g/kg **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS**

No toxic sign reported.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** Rodent Toxicity **SAMPLE:** Spent Bed Dolomite

**MAD:** 10 g/kg

**RESULTS:**

**LD50 (EC50) VALUE**[a] > 10 g/kg **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**

No toxic signs reported.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** Rodent Toxicity **SAMPLE:** Spent Bed Leachate

**MAD:** 10 g/kg or 10 ml/kg

**RESULTS:**

**LD50 (EC50) VALUE**[a] > 10 ml/kg **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**

No toxic signs reported.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Rodent Toxicity SAMPLE: Fly Ash Slurry Sample

MAD: 10 g/kg or 10 ml/kg

RESULTS:

LD50 (EC50) VALUE[a] > 10 ml/kg (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS:

No toxic signs reported.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: Rodent toxicity SAMPLE: Fly Ash Leachate

MAD: 10 g/kg (10 ml/kg)

RESULTS:

LD50 (EC50) VALUE[a] > 10 ml/kg (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS:

No toxic sign reported.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** RAM **SAMPLE:** Slurry (Fly Ash)

**MAD:** 600 µl/ml

**RESULTS:**

**LD50 (EC50) VALUE**[a] 146 µl/ml (ATP fg/cell) **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Low

**COMMENTS:**

Range of EC50s for all parameters = 146 - > 1000 µl.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** RAM **SAMPLE:** #7 - 3 µ Cyclone

**MAD:** 1000 µg/ml

**RESULTS:**

**LD50 (EC50) VALUE**[a] >1000 UG/ML **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:** All parameters

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: RAM SAMPLE: #6 - 1μ filter Cyclone

MAD: 1000 μg/ml

RESULTS:

LD50 (EC50) VALUE[a] 596 μg/ml (ATP fg/cell) (or) AMES RESPONSE

MEC[b]

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Low

COMMENTS: ND in other parameters

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

FBC

ASSAY: RAM SAMPLE: Dolomite

MAD: 600 μl or 1000 μg/ml

RESULTS:

LD50 (EC50) VALUE[a] > 1000 μg/ml (or) AMES RESPONSE

MEC[b]

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS: All parameters

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** RAM **SAMPLE:** Spent bed Dolomite

**MAD:** 600 µl or 1000 µg/ml

**RESULTS:**

**LD50 (EC50) VALUE**[a] >1000 µg/ml **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:** All parameters

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** RAM **SAMPLE(S):** (3) Spentbed leachate
Flyash leachate
Flyash

**MAD:** 600 µl or 1000 µg/ml

**RESULTS:**

**LD50 (EC50) VALUE**[a] > 1000 µg or µl/ml **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:** All parameters

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM**

FBC

**ASSAY:** Salmonella **SAMPLE:** Spent Bed Dolomite

**MAD:** 5 mg/plate

**RESULTS:**

**LD50 (EC50) VALUE**[a] _____ **(or) AMES RESPONSE** Negative

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**

Maximum concentration tested was 5 mg/plate.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM**

FBC

**ASSAY:** Salmonella **SAMPLE:** Fly Ash

**MAD:** 5 mg/plate

**RESULTS:**

**LD50 (EC50) VALUE**[a] _____ **(or) AMES RESPONSE** Negative

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**

Maximum concentration used was 5 mg/plate.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** Salmonella **SAMPLE:** Dolomite

**MAD:** 5 mg/plate

**RESULTS:**

**LD50 (EC50) VALUE**[a] _____ **(or) AMES RESPONSE** Negative

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**

Maximum concentration tested was 5 mg/plate.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** Salmonella **SAMPLE:** 3μ Cyclone Coarse Particulate

**MAD:** 5 mg/plate

**RESULTS:**

**LD50 (EC50) VALUE**[a] _____ **(or) AMES RESPONSE** Positive

**MEC**[b] 2 mg/plate

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Low

**COMMENTS:**

Active in strains TA-1538 and TA-98. Sample was more active without S9 than with S9 at equivalent concentrations.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** FBC

**ASSAY:** Salmonella **SAMPLE:** 1μ Cyclone Fine Particulate

**MAD:** 5 mg/plate

**RESULTS:**

**LD50 [EC50] VALUE**[a] _____ **[or] AMES RESPONSE** Positive

**MEC**[b] 2 mg/plate

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Low

**COMMENTS:**

The sample was active in strains TA-1538 and TA-98.
More activity was obtained in nonactivation tests (-S9)
than in activation tests (+S9) at equivalent concentrations.

[a] For the RAM assay the EC50 for the most responsive parameter will be Noted.

[b] MEC = Minimum effective concentration [If response is positive].

[c] See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM**

CG

**ASSAY:** Marine Algal Assays **SAMPLE:** Holston Separator Liquor

**MAD:** 100 mg/l or 100%

**RESULTS:**

**LD50 [EC50] VALUE**[a] 0.89% (4 days), 0.53% (8 days), 0.41% (12 days) **[or] AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** Filtered

**LEVEL OF TOXICITY**[c] High

**COMMENTS:**

[a] For the RAM assay the EC50 for the most responsive parameter will be listed.

[b] MEC = Minimum effective concentration (if response is positive).

[c] See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: FW Algal Assays SAMPLE: Radian Separator Liquor

MAD: 100 mg/liter or 100%

RESULTS:

LD50 [EC50] VALUE[a] 0.1 - 1% [or] AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] High

COMMENTS:

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: FW Algal Assays SAMPLE: Holston Separator Liquor

MAD: 100 mg/liter or 100%

RESULTS:

LD50 [EC50] VALUE[a] 1.9% (4 days), 0.57% (8 days), 0.53% (12 days) [or] AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR Unfiltered

LEVEL OF TOXICITY[c] High

COMMENTS:

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** CG

**ASSAY:** WI-38 **SAMPLE:** 655 - 5/6

**MAD:** 600 µl or 1000 µg/ml

**RESULTS:**

**LD50 (EC50) VALUE**[a] 1.3 x $10^{-4}$% (Viability Index) **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] (High)

**COMMENTS:**

Cannot interpret concentration relative to established MAD.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** CG

**ASSAY:** WI-38 **SAMPLE:** 590 - 5/6

**MAD:** 600 µl or 1000 µg/ml

**RESULTS:**

**LD50 (EC50) VALUE**[a] 5.1 x $10^{-4}$% (Viability Index) **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] (High)

**COMMENTS:**

Cannot interpret concentration relative to established MAD.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** CG

**ASSAY:** MI-38 **SAMPLE:** Separator Liquor HB - 013

**MAD:** 600 µl/ml or 1000 µg/ml

**RESULTS:**

**LD50 [EC50] VALUE**[a] 7.4 µl/ml (Viability Index) [=] **AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Moderate

**COMMENTS:**

MAD was 60 µl/ml for this sample.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration [if response is positive].

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** CG

**ASSAY:** FW Fish Test **SAMPLE:** Holston Separator Liquor

**MAD:** 100 mg/l or 100%

**RESULTS:**

**LD50 (EC50) VALUE**[a] 0.23% (24 hrs), 0.20% (48 hrs), 0.16% (96 hrs) **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] High

**COMMENTS:**

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** CG

**ASSAY:** Freshwater Fish Test **SAMPLE:** Radian Separator Liquor

**MAD:** 100 mg/liter or 100%

**RESULTS:**

**LD50 (EC50) VALUE**[a] 0.02% (96 hrs) **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] High

**COMMENTS:**

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** CG

**ASSAY:** Daphnia Assay **SAMPLE:** Radian Separator Liquor

**MAD:** 100 mg/liter or 100%

**RESULTS:**

**LD50 (EC50) VALUE**[a] 0.51% (24 hrs) - 0.11% (48 hrs) **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] High

**COMMENTS:**

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

PRIMARY

DATA TRANSITION FORM CG

ASSAY: Salmonella SAMPLE: HB-019 (HAAP cyclone dust)

MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE[a] _____ (or) AMES RESPONSE Negative

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS:
Maximum concentration tested was 5 mg/plate.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

PRIMARY

DATA TRANSITION FORM CG

ASSAY: Salmonella SAMPLE: HB - 018 (HAAP ash)

MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE[a] _____ (or) AMES RESPONSE Negative

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS:
Maximum concentrate tested was 5 mg/plate.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM**

CG

**ASSAY:** Salmonella **SAMPLE:** Coal

**MAD:** 5 mg/plate

**RESULTS:**

**LD50 [EC50] VALUE**[a] _____ **[or] AMES RESPONSE** Negative

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**

Maximum concentration tested was 5 mg/plate.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM**

CG

**ASSAY:** Salmonella **SAMPLE:** HB - 020 (HAAP Coal)

**MAD:** 5 mg/plate

**RESULTS:**

**LD50 [EC50] VALUE**[a] _____ **[or] AMES RESPONSE** Negative

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**

Did not meet preestablished criteria for mutagenicity. However, the report indicated this sample was weakly mutagenic for strains TA-98 and TA-100. A concentration of 10 mg/plate produced less than 2-fold increases over the solvent control for both strains (1.5 - 1.8).

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Salmonella SAMPLE: 590

MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE[a] _____ (or) AMES RESPONSE Positive

MEC[b] 1 mg/plate

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Low

COMMENTS:

This sample was mutagenic for TA-1537, TA-1538, TA-98 and TA-100 only under conditions of activation (+S9).

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Salmonella SAMPLE: 655

MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE[a] _____ (or) AMES RESPONSE Negative

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS:

The sample was tested at a maximum concentration of 5 mg/plate. The submitter's report indicated marginal activity in strain TA-100; however, the increase was less than 1.5 times the spontaneous background and did not meet preestablished criteria for mutagenesis.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM CG

ASSAY: Salmonella SAMPLE: HB-017 (HAAP Separator liquor)

MAD: 5 mg/plate

RESULTS:

LD50 [EC50] VALUE[a] _____ [or] AMES RESPONSE Negative

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS:
The maximum concentration tested was 5 mg/plate.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration [if response is positive].

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM CG

ASSAY: Salmonella SAMPLE: H-032 (HAAP Separator tar)

MAD: 5 mg/plate

RESULTS:

LD50 [EC50] VALUE[a] _____ [or] AMES RESPONSE Positive

MEC[b] 0.01 mg/plate

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] High

COMMENTS:
The sample was mutagenic for strains TA-1537, TA-1538, TA-98 and TA-100.
Acitivity was observed only with S9 activation.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration [if response is positive].

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** CG

**ASSAY:** Rodent Toxicity **SAMPLE:** HAAP Ash

**MAD:** 10 g/kg

**RESULTS:**

**LD50 (EC50) VALUE**[a] > 10 g/kg **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**
No toxic sign reported.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** CG

**ASSAY:** Rodent toxicity **SAMPLE:** HAAP coal

**MAD:** 10 g/kg

**RESULTS:**

**LD50 (EC50) VALUE**[a] > 10 g/kg **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**
No toxic sign reported.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Rodent Toxicity SAMPLE: Coal

MAD: 10 g/kg

RESULTS:

LD50 (EC50) VALUE[a] > 10 g/kg (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable.

COMMENTS:

No toxic signs reported.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: Rodent Toxicity SAMPLE: HAAP Cyclone Dust

MAD: 10 g/kg

RESULTS:

LD50 (EC50) VALUE[a] > 10 g/kg (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS:

No toxic sign reported.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM**

CG

**ASSAY:** Rodent Toxicity **SAMPLE:** HAAP Separator Liquor

**MAD:** 10 g/kg

**RESULTS:**

**LD50 [EC50] VALUE**[a] > 10 g/kg **[or] AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**
No toxic signs reported.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration [if response is positive].

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** CG

**ASSAY:** RAM **SAMPLE:** 590-3

**MAD:** 600 µl/ml

**RESULTS:**

**LD50 (EC50) VALUE**[a] 5.3 µl/ml (ATP fg/cell) **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] High

**COMMENTS:**
Several parameters showed toxicity in this range (5.3 - 13.5 for all parameters).

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** CG

**ASSAY:** RAM **SAMPLE:** 655-3

**MAD:** 600 µl/ml

**RESULTS:**

**LD50 (EC50) VALUE**[a] 21.2 µl/ml (Viab. Index) **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Moderate

**COMMENTS:**
Range of EC50 for all parameters = 21.2 - 101.7.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM CG

ASSAY: RAM SAMPLE: Coal

MAD: 600 µl or 1000 µg/ml

RESULTS:

LD50 (EC50) VALUE[a] > 1000 µg/ml (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS: All parameters

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM CG

ASSAY: RAM SAMPLE: 655 - 5/6

MAD: 600 µl or 1000 µg/ml

RESULTS:

LD50 (EC50) VALUE[a] $1.9 \times 10^{-4}$% viability (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] High

COMMENTS:

Cannot interpret data relative to established MAD.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: 590 - 5/6

MAD: 600 µl or 1000 µg/ml

RESULTS:

LD50 [EC50] VALUE[a] > 4 x $10^{-3}$% (Cell count and protein) [or] AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] (High)

COMMENTS:
Cannot interpret data relative to established MAD.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.
[b]MEC = Minimum effective concentration [if response is positive].
[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

CG

ASSAY: RAM SAMPLE: Coal dust

MAD: 1000 µg/ml

RESULTS:

LD50 [EC50] VALUE[a] > 1000 µg/ml [or] AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS: All parameters not detectable toxicity

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.
[b]MEC = Minimum effective concentration [if response is positive].
[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** CG

**ASSAY:** RAM **SAMPLE:** HAAP ASH HB - 014

**MAD:** 600 µl or 1000 µg/ml

**RESULTS:**

**LD50 (EC50) VALUE**[a] > 300 µg/ml (all parameters) **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Low

**COMMENTS:**
No data > 300 µg/ml; thus cannot interpret.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** CG

**ASSAY:** RAM **SAMPLE:** HAAP COAL HB - 016

**MAD:** 600 µl or 1000 µg/ml

**RESULTS:**

**LD50 (EC50) VALUE**[a] > 1000 µg/ml (all) **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** CG

**ASSAY:** RAM **SAMPLE:** HAAP Cyclone Dust HB - 015

**MAD:** 600 µl or 1000 µg/ml

**RESULTS:**

**LD50 (EC50) VALUE**[a] > 1000 µg/ml (all) **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** CG

**ASSAY:** RAM **SAMPLE:** Separater Tar HB - 026

**MAD:** 600 µl/1000 µg/ml

**RESULTS:**

**LD50 (EC50) VALUE**[a] 350 µg/ml (Viab. Index) **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Low

**COMMENTS:**

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM CG

ASSAY: RAM SAMPLE: Separator Liquor HB - 013

MAD: 600 µl or 1000 µg/ml

RESULTS:

LD50 (EC50) VALUE[a] 12 µl/ml (Viability Index) (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Moderate

COMMENTS:

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM CG

ASSAY: RAM SAMPLE: Cyclone Dust

MAD: 1000 µg/ml

RESULTS:

LD50 (EC50) VALUE[a] > 1000 µg/ml (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS: Non detectable toxicity all parameters

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** CG

**ASSAY:** Grass shrimp test **SAMPLE:** Holston separator liquor

**MAD:** 100 mg/liter or 100%

**RESULTS:**

**LD50 (EC50) VALUE**[a] 0.41% (48 hrs), 0.25% (96 hrs) (or) **AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] High

**COMMENTS:**

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: RAM SAMPLE: R-2-15

MAD: 600 µl or 1000 µg/ml

RESULTS:

LD50 (EC50) VALUE[a] > 600 µl/ml (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS: All parameters

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: RAM SAMPLE: M-2-15

MAD: 600 µl or 1000 µg/ml

RESULTS:

LD50 (EC50) VALUE[a] ~ 400 µl/ml (Vlab. Index) (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Low

COMMENTS:

EC50 estimated from raw data.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: RAM SAMPLE: N-2-15

MAD: 600 µl or 1000 µg/ml

RESULTS:

LD50 (EC50) VALUE[a] ~ 175 µl/ml (ATP) (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Low

COMMENTS:

EC50 estimated from raw data showing EC50 >60 and <200.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: RAM SAMPLE: L-2-15

MAD: 600 µl or 1000 µg/ml

RESULTS:

LD50 (EC50) VALUE[a] ~ 300 µl/ml (ATP) (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Low

COMMENTS:

EC 50 Estimated from raw data.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: CHO SAMPLE: R-2-15

MAD: 600 µl/ml

RESULTS:

LD50 (EC50) VALUE[a] ~ 37 µl/ml (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Moderate

COMMENTS:

EC50 estimated from raw data.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: CHO SAMPLE: N 2-15

MAD: 600 µl/ml

RESULTS:

LD50 (EC50) VALUE[a] ~ 75 µl/ml (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Low

COMMENTS:

EC50 estimated from raw data.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: CHO SAMPLE: M-2-15

MAD: 600 µl/ml

RESULTS:

LD50 (EC50) VALUE[a] > 200 µl/ml (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Low

COMMENTS:

200 µl/ml was highest concentration used. Thus could not determine ED50 in context of MAD.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: CHO SAMPLE: L-2-15

MAD: 600 µl/ml

RESULTS:

LD50 (EC50) VALUE[a] ~ 60 µl/ml (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Low

COMMENTS:

EC50 estimated from raw data. Value slightly > 60 µl/ml.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** WE

**ASSAY:** Marine Algae **SAMPLE:** F-2

**MAD:** 100%

**RESULTS:**

**LD50 (EC50) VALUE**[a] 56 - 75% **(or) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Moderate

**COMMENTS:**

LC50 - 70% 90% mortality with 100% effluent.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM WE

ASSAY: WI-38 SAMPLE: R-2-15

MAD: 600 µl/ml

RESULTS:

LD50 (EC50) VALUE[a] > 600 µl/ml (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable.

COMMENTS: All parameters

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM WE

ASSAY: WI-38 SAMPLE: M-2-15

MAD: 600 µl/ml

RESULTS:

LD50 (EC50) VALUE[a] > 600 µl/ml (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS: All parameters

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** WE

ASSAY: WI-38 SAMPLE: N-2-15

MAD: 600 µl/ml

RESULTS:

LD50 [EC50] VALUE[a] ~ 200 µl/ml (ATP) [or] AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Low

COMMENTS:

EC50 estimated from raw data.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** WE

ASSAY: WI-38 SAMPLE: L-2-15

MAD: 600 µl/ml

RESULTS:

LD50 [EC50] VALUE[a] ~ 300 µl/ml (ATP) [or] AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Low

COMMENTS:

EC50 estimated from raw data showing EC50 >200 µl and <600 µl/ml.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM WE

ASSAY: Grass shrimp SAMPLE: L-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] - >100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS:

Nontoxic at 100%.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM WE

ASSAY: Grass shrimp SAMPLE: N-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] 10 - 32% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Moderate

COMMENTS:

LC50 about 25%

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM**

WE

**ASSAY:** Grass Shrimp **SAMPLE:** S-2

**MAD:** 100%

**RESULTS:**

**LD50 (EC50) VALUE**[a] _____ - >100% _____ **(+/-) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] ND

**COMMENTS:**

20% toxicity at 100% effluent

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM**

WE

**ASSAY:** Grass shrimp **SAMPLE:** U - 2

**MAD:** 100%

**RESULTS:**

**LD50 (EC50) VALUE**[a] _____ - >100% _____ **(+/-) AMES RESPONSE** _____

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] ND

**COMMENTS:**

Nontoxic at 100%.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass shrimp SAMPLE: W-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] 18 - 32% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Moderate

COMMENTS:
LC50 - 25% effluent.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Grass shrimp SAMPLE: X-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] - ≥100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS: Not detectable

Nontoxic at 100%

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM WE

ASSAY: Grass shrimp SAMPLE: A-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] 32 - 18% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Moderate

COMMENTS:

LC50 at about 25% effluent conc.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM WE

ASSAY: Grass shrimp SAMPLE: B-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] >100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS:

No toxicity at 100%.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM WE

ASSAY: Grass shrimp SAMPLE: C-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] 100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] LOW

COMMENTS:

100% = only 56% mortality.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM WE

ASSAY: Grass shrimp SAMPLE: F-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] >100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS:

No toxicity at 100%.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** WE

ASSAY: Grass shrimp SAMPLE: G-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] - >100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS:
Only 20% mortality at 100% effluent.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**
**DATA TRANSITION FORM** WE

ASSAY: Grass shrimp SAMPLE: K-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] - >100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS:
Nontoxic at 100%

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM WE

ASSAY: Marine Fish SAMPLE: W-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] - >100% / (or) AMES RESPONSE

MEC[b]

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS: Not detectable

Nontoxic at 100%.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM WE

ASSAY: Marine Fish SAMPLE: W-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] 32 - 56% (or) AMES RESPONSE

MEC[b]

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Moderate

COMMENTS:

56% effluent produced 100% lethality.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY

DATA TRANSITION FORM WE

ASSAY: Marine Fish SAMPLE: U-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] - >100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

Nontoxic at 100%.

COMMENTS: Not detectable

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY

DATA TRANSITION FORM WE

ASSAY: Marine Fish SAMPLE: C-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] 56 - 75% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Moderate

COMMENTS:
LC50 - 70% 100% toxic at 100% effluent.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: F-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] - >100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS:

Nontoxic at 100%

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: G-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] - >100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS: Non toxic

40% toxicity at 100%

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: K-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] - >100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS:

Nontoxic

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Marine Fish SAMPLE: L-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] 100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Low

COMMENTS:

LC50 = 100%.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM WE

ASSAY: Marine Fish SAMPLE: N-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] 32 - 56% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Moderate

COMMENTS:

LC50 - 40% Complete lethality at 100% effluent.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM WE

ASSAY: Marine Fish SAMPLE: S-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] >100% (or) AMES RESPONSE _____

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS: Not detectable

20% mortality at 100% effluent.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

/ PRIMARY

DATA TRANSITION FORM WE

ASSAY: Marine Fish SAMPLE: B-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] - >100% (or) AMES RESPONSE

MEC[b]

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND

COMMENTS:

Nontoxic at 100%.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

/ PRIMARY

DATA TRANSITION FORM WE

ASSAY: Marine Fish SAMPLE: A-2

MAD: 100%

RESULTS:

LD50 (EC50) VALUE[a] 56 - 75% (or) AMES RESPONSE

MEC[b]

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Moderate

COMMENTS:

LC50 ~ 60% effluent

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Salmonella

MAD: 5 mg/plate

SAMPLE: L-2-5 R-2-5 U-2-5 X-2-5
M-2-5 S-2-5 V-2-5 Y-2-5
N-2-5 T-2-5 W 2-5 Z-2-5

RESULTS:

LD50 (EC50) VALUE[a] _____ (or) AMES RESPONSE Negative

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS:
All samples were tested at a maximum value of 1.0 ml/plate.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive)

[c]See Table 1 for definition of toxicity levels.

PRIMARY
DATA TRANSITION FORM

WE

ASSAY: Salmonella

SAMPLE: P-2-5

MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE[a] _____ (or) AMES RESPONSE Negative

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS:
Sample was tested at a maximum concentration (volume) per plate of 500 µl.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**PRIMARY**

**DATA TRANSITION FORM** WE

**ASSAY:** Salmonella **SAMPLE:** C-2-5 G-2-5
D-2-5 H-2-5
**MAD:** 5 mg/plate E-2-5 J-2-5
F-2-5 K-2-5

**RESULTS:**

**LD50 (EC50) VALUE**[a] _____ **(or) AMES RESPONSE** Negative

**MEC**[b] _____

**APPROXIMATE CONCENTRATION FACTOR** 0

**LEVEL OF TOXICITY**[c] Nondetectable

**COMMENTS:**

All samples employed 1.0 ml as the maximum concentration/plate.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY

DATA TRANSITION FORM

WE

ASSAY: Salmonella SAMPLE: B2

MAD: 5 mg/plate

RESULTS:

LD50 (EC50) VALUE[a] _____ (or) AMES RESPONSE Negative

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] Nondetectable

COMMENTS:

Maximum amount applied per plate was 1.0 ml.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

PRIMARY

DATA TRANSITION FORM

WE

ASSAY: Salmonella SAMPLE: A-2-

MAD: 5 mg/kg

RESULTS:

LD50 (EC50) VALUE[a] _____ (or) AMES RESPONSE Negative

MEC[b] _____

APPROXIMATE CONCENTRATION FACTOR 0

LEVEL OF TOXICITY[c] ND Nondetectable

COMMENTS:

Maximum amount added per plate was 1.0 ml.

[a]For the RAM assay the EC50 for the most responsive parameter will be listed.

[b]MEC = Minimum effective concentration (if response is positive).

[c]See Table 1 for definition of toxicity levels.

**TECHNICAL REPORT DATA**
*(Please read Instructions on the reverse before completing)*

| 1. REPORT NO. | 2. | 3. RECIPIENT'S ACCESSION NO. |
|---|---|---|
| EPA-600/7-80-079 | | |

| 4. TITLE AND SUBTITLE | 5. REPORT DATE |
|---|---|
| Level 1 Biological Testing Assessment and Data Formatting | April 1980 |
| | 6. PERFORMING ORGANIZATION CODE |
| 7. AUTHOR(S) | 8. PERFORMING ORGANIZATION REPORT NO. |
| David J. Brusick | |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS | 10. PROGRAM ELEMENT NO. |
| Litton Bionetics, Inc.<br>5516 Nicholson Lane<br>Kensington, Maryland 20795 | INE623 |
| | 11. CONTRACT/GRANT NO. |
| | 68-02-2681, Task 401 |
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15. SUPPLEMENTARY NOTES IERL-RTP project officer is Raymond G. Merrill, Mail Drop 62, 919/541-2557.

16. ABSTRACT The report gives results of an examination of data from three pilot studies including water effluent, fluidized-bed combustion, and coal gasification samples, using a scheme to compare health effects and ecological bioassay assessment data. The scheme is based on the assumption that each test method has a maximum dose (concentration) which can be reliably applied and that effects based on fractions of the maximum applicable dose (MAD) can be designated as degrees of toxicity. The levels of toxicity based on fractions of the MAD are given as high, moderate, low, and nondetectable (i.e., no effect detected at the MAD).

17. KEY WORDS AND DOCUMENT ANALYSIS

| a. DESCRIPTORS | b. IDENTIFIERS/OPEN ENDED TERMS | c. COSATI Field/Group |
|---|---|---|
| Pollution | Pollution Control | 13B |
| Coal Gasification | Stationary Sources | 06A |
| Bioassay | | 06T |
| Toxicity | | |
| Waste Water | | |
| Combustion | | 21B |
| Fluidized Bed Processing | | 13H, 07A |

| 18. DISTRIBUTION STATEMENT | 19. SECURITY CLASS *(This Report)* | 21. NO. OF PAGES |
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